Tuesday September 4th – Archibald / Campbell
Cleaner Fish Disease 1 & 2
Moderator – Gustavo Ramirez-Paredes (Ridgeway Biologicals)

2:15 PM	Cleaner Fish 1	Gulla - Infectious Diseases of Cleaner Fish in Norway
2:30 PM		**Scholz - Cleaner Fish: Emerging Diseases and Biosecurity Implications**
2:45 PM		Midtlyng - "Cleanerfish Bank" and "Kindergartens" – New Management Tactics to Maintain Control of Salmon Lice Without Chemotherapeutic or Handling Interventions
3:00 PM		**Refreshments**
3:15 PM	Cleaner Fish 2	Sandlund - Screening for Pathogens in Wild Goldsinny Wrasse (*Ctenolabrus rupestris*), an Important Cleaner Fish in Norwegian Aquaculture
3:30 PM		**Chakraborty - Infection Model Development and Immunization of Lumpfish (*Cyclopterus lumpus*) Against *Aeromonas salmonicida***
3:45 PM		Ramirez-Paredes - Efficacy of Multivalent Autogenous Vaccines Against Atypical Furunculosis and Vibriosis in Scottish Ballan Wrasse (*Labrus bergylta*)
4:00 PM		Powell - Amoebic Gill Disease in Ballan Wrasse (*Labrus bergylta*) Juveniles and Its Control by UV Irradiation.
4:15 PM		**Papadopoulou - Bath Challenge Model Against Atypical *Aeromonas salmonicida* in Farmed Ballan Wrasse (L. *Bergylta*)**
4:30 PM		**Buba - Phenotypic and Genotypic Characterisation of Atypical *Aeromonas salmonicida* in Ballan Wrasse (*Labrus bergylta,* Ascanius 1767)**
4:45 PM		O'Brien - Pathobiology of an *Exophiala* sp. Disease Event From Aquaculture Reared Lumpfish (*Cyclopterus lumpus*) in Newfoundland and Labrador, Canada
5:00 PM		**Scholz - Experimental Investigations Into Ranavirus (Iridoviridae) Infections in Lumpfish (*Cyclopterus lumpus*).**

Infectious Diseases of Cleaner Fish in Norway

Snorre Gulla * and Duncan J. Colquhoun

Norwegian Veterinary Institute, Fish Health Research group, Ullevålsveien 68, 0454 Oslo, Norway snorre.gulla@vetinst.no, duncan.colquhoun@vetinst.no

The use of cleaner fish has expanded dramatically in Norwegian salmon farming in recent years due to increasing chemoterapeutant resistance in salmon lice. While wild-caught wrasse species dominated initially, cleaner fish used in Norway today predominantly consist of farmed lumpsucker, which tolerate lower water temperatures. In 2017, just over 50 million cleaner fish were used (official statistics) in Norway, and estimates for 2018 range around 60 million. These high numbers reflect the short life expectancy of cleaner fish after stocking in salmon farms.

A large proportion of cleaner fish losses are related to infectious diseases. While bacterial diseases are known to play a leading role, the significance of viral infections (such as Lumpfish Flavivirus) and the various parasites infecting these fish species remains to be established. Bacterial pathogens of cleaner fish regularly detected in Norway include 'atypical' *Aeromonas salmonicida*, *Pasteurella* sp., *Pseudomonas anguilliseptica*, *Moritella viscosa*, *Tenacibaculum* spp. and *Vibrio anguillarum*/*ordalii*, in addition to a range of other *Vibrio* species. *A. salmonicida* subsp. *salmonicida* and *Paramoeba perurans*, both serious pathogens of Atlantic salmon, have also been sporadically detected in cleaner fish. While vaccination of farmed Norwegian cleaner fish against some bacterial pathogens may have contributed towards some mitigation of losses, much optimisation work remains in terms of cleaner fish vaccinology.

The high and relatively rapid mortalities experienced amongst cleaner fish today undoubtedly represent a major animal welfare concern, raising a legitimate question mark over the ethicality of current cleaner fish practices. Current and historic trends from cleaner fish diagnostics and research performed at the Norwegian Veterinary Institute will be presented, with a focus on relevant bacterial pathogens.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)

Cleaner Fish: Emerging Diseases and Biosecurity Implications.

Felix L Scholz[1,2*], Hamish D Rodger[1], Ian O'Connor[2], Luca Mirimin[2], Neil M Ruane[3], Mar Marcos-López[1], Susie O Mitchell[1] and Eugene MacCarthy[2]

[1] FishVet Group Ireland, Oranmore, Co. Galway, Ireland felix.scholz@fishvetgroup.com, hamish.rodger@fishvetgroup.com, mar.marcos-lopez@fishvetgroup.com, susie.mitchell@fishvetgroup.com
[2] Marine and Freshwater Research Center, Galway-Mayo Institute of Technology, Galway, Ireland Ian.OConnor@gmit.ie, luca.mirimin@gmit.ie, eugene.mccarthy@gmit.ie
[3] Fish Health Unit, Marine Institute, Oranmore, Co. Galway, Ireland neil.ruane@marine.ie

Sea lice, *Lepeophtheirus salmonis*, are endemic in European Atlantic salmon farming, limiting the growth of the industry and compromising its sustainability in some regions. Chemical treatments are expensive, their use is in part restricted by legislation and resistances are emerging in sea lice, making the development of non-medicinal solutions for sea lice control a priority for the industry. One approach that has increased in recent years is the stocking of lumpfish (*Cyclopterus lumpus*) and wrasse species (*Labridae*) as cleaner fish. Wrasse species are mostly wild caught while lumpfish are farmed using wild caught broodstock. Mortalities of cleaner fish at sea have been high, partly unexplained and largely attributed to infectious diseases. To expand our limited knowledge on pathogens of cleaner fish and potential biosecurity risks to cohabited Atlantic salmon, the health status of Irish cleaner fish populations at sea and in hatcheries was monitored over the course of three years. Multiple pathogens identified in this study were not previously known to infect cleaner fish species and findings included significant pathogens of Atlantic salmon, such as *Neoparamoeba perurans* for which interspecies transmission between lumpfish and salmon has been proven. Three pathogens of salmon were described in cleaner fish species for the first time: piscine myocarditis virus (PMCV) in wrasse and *Piscirickettsia salmonis* and *Exophiala salmonis* in lumpfish. The first case of microsporidiosis due to *Tetramicra brevifilum*, a significant pathogen of turbot (*Scophthalmos maximus*), was described in lumpfish. A new species of ranavirus, which has been isolated from clinically healthy lumpfish in Iceland, Scotland and the Faroe Islands, was isolated from lumpfish fry experiencing mortality. The findings of this study indicate potential biosecurity risks associated with the use of cleaner fish with implications for practices such as reuse of cleaner fish, moving them between pens and the use of wild caught stock. An overview of emerging pathogens of concern will be presented and implications discussed.

This research is funded by the Irish Research Council (Employment Based Postgraduate Program), FishVet Group Ireland and in part by Bord Iascaigh Mhara.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)

“ Cleanerfish Bank ” and “ Kindergartens ” – New Management Tactics to Maintain Control of Salmon Lice Without Chemotherapeutic or Handling Interventions.

Paul J. Midtlyng[1,2 *] Sturla Romstad[3] and Arnfinn Aunsmo[1,4]

[1] School of Veterinary Medicine, Norwegian University of Life Sciences, Ullevalsveien 72, N 0104 Oslo, Norway paul.midtlyng@nmbu.no
[2] Aquamedic AS, N-0349 Oslo, Norway
[3] Aqua Kompetanse AS, N-7770 Flatanger, Norway sturla-r@online.no
[4] Masoval Fiskeoppdrett AS, N-7260 Sistranda, Norway arnfinn@masoval.no

Until quite recently, successful control of salmon louse (*Lepeophtheirus salmonis*) infestations in Norwegian farmed salmon became increasingly difficult due to development of multi-resistance to the active ingredients used in licensed medicines. The critical situation stimulated rapid development and use of various non-medicinal methods for removing lice, including equipment for mechanical brushing, use of low-pressure washing, short-term warm water exposure, or use of wellboats for long-term freshwater baths. However, all these methods require rather extensive handling operations that cause considerable stress to the fish, resultant mortality, and high labor and equipment costs. Aquaculture veterinarians and their clients in mid-Norway have, therefore, developed a number of new tactical means for use in salmon louse control, that require neither the use of chemotherapy nor handling.

A “cleanerfish bank” means a repository of juvenile cleanerfish on-site, where these fish are fed and cared for while awaiting deployment into farmed salmon cages. This allows for flexibility in delivery time and number relative to salmon sea transfer and the most reproductive season of the lice and enables maximally flexible reaction to increasing numbers of mobile and mature salmon lice by rapidly moving the waiting cleanerfish into cages where and when the need for their workforce is most urgent.

“Kindergartens” are sea transfer sites situated so that smolts and post-smolts are maximally sheltered from copepodid challenge. Typically, the sites are placed in fjords with significant freshwater influx in spring and early summer, or with minimal surface currents likely to carry louse larvae from upstream areas with high density of pre-harvest size salmon. “Kindergarten” sites are typically used for spring entry smolts for 6-8 months while the total biomass is low. The fish will normally be moved to sites with larger carrying capacity and currents carrying infective louse larvae in late autumn (S1 smolts) or late spring (S0 smolts).
Recent field results from mid-Norway will be presented to illustrate how the louse levels on farmed salmon have been gradually and substantially reduced. In our opinion, this is in large parts due to employment of the new fish health management tools described.

Conference Session Designation: (Aquatic Animal Health Management)
Presentation Format: (Oral)

Screening for Pathogens in Wild Goldsinny Wrasse (*Ctenolabrus rupestris*), an Important Cleaner Fish in Norwegian Aquaculture

Nina Sandlund*[1], Stein Mortensen[1], Egil Karlsbakk[1,2], Åge Høines[1], Bjørn Olav Kvamme[1]

[1] Institute of Marine Research, P.O.Box 1870, Nordnes, 5817 Bergen, Norway ninasa@hi.no
[2] Department of Biology, University of Bergen, P.O.Box 7800 5020 Bergen, Norway

The development of resistance of salmon lice (*Lepeophtheirus salmonis*) towards various chemical treatments calls for alternative delousing methods in salmonid aquaculture. The use of cleaner fish as biological control of sealice in Norwegian salmonid farming has increased steadily over the last decade. Different species of wrasse and lumpfish (*Cyclopterus lumpus*) are used. All lumpfish and some ballan wrasse are farmed, but most wrasse is wild caught and goldsinny wrasse (*Ctenolabrus rupestris*) is the most important species.
The loss of wrasse in net pens may be high, from escapes or disease and mortality.
In 2017 almost 28 million wild caught wrasses were used as cleaner fish in Norway. Large quantities of fish are transported between different regions in the country and approximately 1 million were imported from Sweden. The health status of such wild caught fish is mostly unknown, so there is a potential for the wrasse to act as disease vectors. Currently, we gathered information on this, and one approach is the screening wild wrasse for pathogens in the recipient areas. These areas had high densities of fish farms using high amounts of imported wrasse. Fish from other areas with lower densities of aquaculture farms were also sampled. One aim was to better understand the potential risk for disease transfer from wild wrasse to salmon and rainbow trout in the net pens (e.g. VHSV). The main aim was to reveal evidence for any past introductions, from quantitative (prevalence) and qualitative studies (genotypes).
In 2017, almost 1000 goldshinny wrasse were examined for the presence of various pathogens. The results showed a high prevalence of Nucleosporidae gen. sp. in the gills and a more variable presence of *Aeromonas salmonicida* ssp. in the kidney and Betanodavirus in brain. VHSV was not detected.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: **(** Oral)

Infection Model development and Immunization of Lumpfish (*Cyclopterus lumpus*) Against *Aeromonas salmonicida*

Setu Chakraborty[1*], Trung Cao[1], Hajarooba Gnanagobal[1], Ahmed Hossain[1], Danny Boyce[2], and Javier Santander[1]

[1] Marine Microbial Pathogenesis and Vaccinology Laboratory, Department of Ocean Sciences, Memorial University of Newfoundland, Ocean Sciences Centre, 0 Marine Lab Rd, Logy Bay, NL A1K 3E6, Canada schakraborty@mun.ca jsantander@mun.ca

[2] Joe Brown Aquatic Research Building, Department of Ocean Sciences, Memorial University of Newfoundland, Ocean Sciences Centre, 0 Marine Lab Rd, Logy Bay, NL A1K 3E6, Canada dboyce@mun.ca

One of the major current health challenges for the aquaculture industry is sea-lice (*Lepeophtheirus salmonis*) infestation. Lumpfish (*Cyclopterus lumpus*), a native fish to Canada, is a biological delousing agent. The use of cleaner fish is attractive because they can reduce the use of chemo therapeutants and, in addition, it is less stressful to the fish. The number of cleaner fish used by the salmon farming industry has increased exponentially since 2008, and it is estimated that 50 million lumpfish will be required by 2020. The cleaner fish delouse the salmon skin by eating the parasite, but also ingest other potential pathogens that may be transmitted by sea lice. Cleaner fish pathogens post sea transfer are currently a major global challenge, especially bacterial infections caused by *Aeromonas salmonicida* among other pathogens. In this study, we followed the *A. salmonicida* infection in lumpfish to establish a vaccine challenge model. Groups of 120 fish where intraperitoneally (i.p.) injected with different doses of *A. salmonicida* ranges from 10^1 to 10^5 cells per fish. Samples of blood, head-kidney, spleen, and liver were collected at different time points. *A. salmonicida* was detected after 5 days post-infection in the head kidney and later in the rest of the tissues. *A. salmonicida* killed lumpfish in a dose-dependent fashion and the lethal dose 50 (LD_{50}) was estimated at 10^2 CFU/ml. Also, we evaluate *A. salmonicida* iron uptake outer membrane proteins (IROMPs), outer membrane proteins (OMPs), *Aeromonas salmonicida* bacterin grown under iron-limited conditions as a vaccine and compared to a commercial vaccine preparation. Groups of 100 fish where intraperitoneally (i.p.) immunized and boost 4 weeks post prime-immunization. Samples of blood, head-kidney, spleen, and liver were collected at different time points. Twenty-one weeks post prime-immunization the fish were i.p. challenged with a high dose of the *A. salmonicida* (10^7 cells per fish) to evaluate vaccine efficacy. We found that fish immunized with *A. salmonicida* OMPs develop a toxic shock-like after boost immunization. Challenge assays showed that the different vaccine formulations conferee similar levels of protection.

Conference Session Designation: (Immunology Vaccines / Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)

Efficacy of Multivalent Autogenous Vaccines Against Atypical Furunculosis and Vibriosis in Scottish Ballan Wrasse (*Labrus bergylta*)

J Gustavo Ramirez-Paredes[1,2*], Athina Papadopoulou[1], Sean J Monaghan[1], Rimmer G[3], Louise Smith[3], Tim Wallis[2], Carolina Gutierrez[4], David Cockerill[4], Andrew Davie[1], David Verner-Jeffreys[3], Herve Migaud[1], Alexandra Adams[1]

[1] Institute of Aquaculture, University of Stirling, Stirling, Scotland U.K. jgr1@stir.ac.uk ap50@stir.ac.uk sjm27@stir.ac.uk alexandra.adams@stir.ac.uk
[2] Ridgeway Biologicals, Compton, Berkshire, England U.K. gus.ramirez-paredes@ridgewaybiologicals.co.uk tim.wallis@ridgewaybiologicals.co.uk
[3] Cefas, Weymouth Laboratory, Weymouth, England U.K. david.verner-jeffreys@cefas.co.uk
[4] Marine Harvest Scotland, Fort William, Scotland, U.K. karol.gr87@gmail.com

Recent bacteriological surveys of the Scottish ballan wrasse industry have indicated that atypical *Aeromonas salmonicida* (a*As*) and *Vibrionaceae*-related bacteria are predominant diagnostic findings during natural outbreaks of disease. Some of these bacteria are very likely to have a role in the high mortalities experienced on production sites, and so hatcheries are at present vaccinating with autogenous vaccines containing antigens derived from a wide range of these putative pathogens. The use of these broad spectrum autogenous vaccines has proven to effective controlling mortalities in the field. However information regarding the level of protection conferred per component and the virulence of the strains used as antigens remains to be elucidated. In this study, infections via intra peritoneal injection were performed to investigate the virulence of three subtypes of a*As* and three isolates of *Vibrio splendidus* (*Vs*) in pre-deployment wrasse. While the experimental infections with a*As* successfully reproduced the clinical presentation atypical furunculosis, the i.p. injection of 10^9 to 10^{10} cfu/fish of *Vs* did not cause significant mortalities or clinical signs of vibriosis over a period of 7 days. An *in vivo* challenge model was established with a*As* and used to assess the efficacy of a commercial autogenous vaccine. Specific antibody responses to a*As* and bacterial loads of *aAs* in vaccinated and challenged fish were analysed by ELISA and qPCR as correlates of protection. The vaccine provided 79% and 20% relative percent survival (RPS) against experimental homologous challenge with $1x10^7$ and $1x10^8$ cfu a*As vapA* V B4 / fish, respectively, and 95% and 91% RPS against $1x10^6$ and $1x10^7$ cfu a*As vapA* V B2 / fish, respectively after an immunisation period of 700 degree days.

Conference Session Designation: (Immunology-Vaccines or Cleaner Fish)
Presentation Format: (Oral)

Amoebic Gill Disease in Ballan Wrasse (*Labrus Bergylta*) Juveniles and its Control by UV Irradiation.

Mark D. Powell[1,2*], Anders Lepperød[2], Herman Kvinnsland[3] Aina-Charlotte Wennberg[4]

[1] Institute of Marine Research, Bergen Norway Mark.Powell@HI.no
[2] Department of Biosciences, University of Bergen, Bergen Norway Mark.Powell@uib.no
[3] Present address: Fiskehelse og miljø AS, Haugesund, Norway herman@fom-as.no
[4] Norwegian Institute for Water Research, Gaustadaleen 21, Oslo, Norway aina.charlotte.wennberg@niva.no.

Amoebic gill disease caused by *Neoparmaoeba perurans* continues to be a significant challenge for the producers of cleaner fish (such as ballan wrasse *Labrus bergylta*, lumpfish *Cyclopterus lumpus*) in land-based pump-ashore hatcheries as well as in open sea cages. Since the produced fish are destined for stocking with highly AGD-susceptible Atlantic salmon as a biological control of sea lice, it remains imperative that they are not potential vectors of AGD in the sea cage environment. *Neoparamoeba perurans* re-isolated from fish after a challenge with a clonal strain, were maintained in liquid culture. Amoebae were exposed to ultraviolet radiation or ozone at different doses for varying periods of time. The subsequent morphology and growth characteristics of the amoebae cultures was monitored using MPN methods. UV appeared to have significant effects on inhibiting growth at most of the doseages used, although amoebae survived. In a laboratory challenge study, ballan wrasse juveniles were exposed to 100 cells L^{-1} of *Neoparamoeba perurans* trophozoites either irradiated with 0, 2 or 20 mJ cm^{-2} UV. Over the subsequent 6 week period, AGD developed only in the 0 UV group. AGD-affected wrasse developed epithelial hyperplasia, characteristic of AGD, in individual filaments with large numbers of unaffected filaments exhibiting a normal respiratory epithelium. In the ballan wrasse, hyperplasia associated with AGD lesions were characterized by increased numbers of mucous cells and an infiltration of eosinophilic granule cells. These studies suggest that UV irradiation of water at doses exceeding 2 mJ cm^{-2} is sufficient to inhibit infection of ballan wrasse with *Neoparamoeba perurans* and the subsequent development of AGD.

Conference Session Designation: (Gill Health or Cleaner Fish Heath)
Presentation Format: **(** Oral)

Bath Challenge Model Against Atypical Aeromonas Salmonicida in Farmed Ballan Wrasse (*L. Bergylta*)

Athina Papadopoulou[1*], Kathryn Garvey[1], Jose G Ramirez-Paredes[1,2], Sean J Monaghan[1], Andrew Davie[1], Tom Hill[3], Ioanna Katsiadaki[3], David Verner-Jeffreys[3], Carolina Gutiérrez[4], Tim Wallis[2], Antonios Chalaris[5], Alastair Barge[5], Herve Migaud[1], Alexandra Adams[1]

[1] Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling FK9 4LA, UK athina.papadopoulou@stir.ac.uk

[2] Ridgeway Biologicals Ltd, Units 1-3 Old Station Business Park, Compton, Nr Newbury G20 6NE UK gus.ramirez-paredes@ridgewaybiologicals.co.uk, tim.wallis@ridgewaybiologicals.co.uk

[3] Centre for Environment, Fisheries and Aquaculture Science, Barrack Road, The Nothe Weymouth, Dorset, DT4 8UB UK tom.hill@cefas.co.uk, ioanna.katsiadaki@cefas.co.uk

[4] Marine Harvest, Ltd, Stob Ban House, Glen Nevis Business Park, Forth William, PH33 6RX UK

[5] Otterferry Seafish Ltd, Tighnabruaich, Argyll, PA21 2DH UK a.e.chalaris@stir.ac.uk, a.barge@otterferry.com

Ballan wrasse (*Labrus bergylta*) is commercially farmed and deployed as cleaner fish in Atlantic salmon cages as an environmentally friendly approach to delousing. Atypical *Aeromonas salmonicida;* a*As*, representing an important bacterial pathogen of *L. bergylta*, and other potential pathogens were isolated during outbreaks at hatcheries and/or cage sites in Scotland between 2016 and 2017. The pathogenicity and virulence of these routinely recovered bacteria including one isolate from Norway were assessed on juvenile (approx. 2 g) farmed Ballan wrasse by bath exposure, which enabled the development of a bath challenge model against a*As*. Juvenile Ballan wrasse (n= 50) were exposed to four bacteria species – a*As*, *Aliivibrio salmonicida*, *Photobacterium indicum* and *Vibrio anguillarum*; Scottish and Norwegian isolates – two strains of each species in duplicate (8 groups x 2 = 16; 2 controls; 18 tanks total) at an OD 1.0 (10^5 – 10^7 cfu/ml bacterial strain dependant) in 5 l sea water (33ppt) for 4 h at 15°C in static conditions. Duplicate groups of control fish were exposed to sterile sea water. The fish were then split into 18 tanks and monitored for up to 22 days. Moribund and diseased fish were examined for gross pathological changes and samples were taken for bacteriology, histopathology and molecular assessment. Ballan wrasse juveniles were susceptible to a*As* which was in contrast with the Vibrionaceae tested in this study. Notably, differential virulence was observed for a*As* subtype V - sub pulsotypes. Greater cumulative mortalities of 52 and 60 % were recorded when fish were challenged with a*As* sub pulsotypes B2 in contrast to 20% for B4. Furthermore, 4% mortality was observed for a Scottish isolate of *V. anguillarum;* while no mortalities were recorded for a Norwegian isolate of *V. anguillarum* or *Allivibrio salmonicida* and *P. indicum*. To our knowledge this is the first report of a successful a*As* bath challenge model for juvenile Ballan wrasse. This study provides an important foundation for future studies on vaccine efficacy, protection and immunocompetence at this developmental stage.

Program Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentaton: (Yes)

Phenotypic and Genotypic Characterisation of Atypical *Aeromonas Salmonicida* in Ballan Wrasse (*Labrus Bergylta*, Ascanius 1767)

Elizabeth Buba*[1, 2], Athina Papadopoulou[1], Kerry L. Bartie[1], Michael Bekaert[1], Jose G. Ramirez-Paredes[3], Tim Wallis[3], Carolina Gutierrez[4], Lindsay Sherrif [4], Paul. Featherstone[4], David Cockerill[4], Andrew Davie[1], Andrew Desbois[1], Herve Migaud[1], Alexandra. Adams[1]

[1] Institute of Aquaculture, University of Stirling, Stirling FK9 4LA U
elizabeth.buba1@stir.ac.uk
[2] College of Veterinary Medicine, Federal University of Agriculture Makurdi, Nigeria
[3] Ridgeway Biologicals Ltd, Compton, Newbury RG20 6NE UK
[4] Marine Harvest Ltd, Stob Ban House, Fort William, PH33 6RX UK

Cleaner fish, Ballan wrasse (*Labrus bergylta*) and lumpsucker (*Cyclopterus lumpus*) are used as an alternative approach for effective removal of sea lice from Atlantic salmon in aquaculture. Although both species are susceptible to infection by atypical *Aeromonas salmonicida* (a*As*), little is known about the diversity of a*As*. The aim of this study was to characterise 87 a*As* isolates from cleaner fish in Scotland and to compare these to a*As* from other fish species. Phenotypic characterisation of the a*As* isolates was initially performed to compare 35 representative isolates using conventional bacterial identification methods and a profiling system (BIOLOG GEN III) composed of 94 biochemical assays. Genotyping methods based on a PCR assay for the *virulence array protein* gene (*vapA*; A-layer), macro-restriction analysis using pulsed-field gel electrophoresis (PFGE) and plasmid sequencing was also conducted on the isolates. Phenotypically, the a*As* isolates resembled translucent, circular, convex colonies with or without brown diffusible pigmentation and were found to be non-motile, oxidase positive and Gram-negative coccobacilli or short rods under microscopic examination. The BIOLOG GEN III panel showed variability in 22 biochemical tests, of which two tests separated the UK a*As* isolates into two groups according to the *vapA* type; a minor group A (*vapA* type VI; n= 12 isolates) associated with both wrasse and lumpsucker fish species and a major group B (*vapA* type V; n= 75) confined to the wrasse host. Band analysis of the PFGE profiles revealed 10 pulsotypes from six sites in Scotland that also clustered according to the two *vapA* types. The major PFGE cluster B could be further sub-divided into nine pulsotypes (B1 - B9), with B2 pulsotype being predominant (n= 56) at four different sites. Plasmid profiling indicated the existence of multiple plasmids ranging in size from 5 to 155 kb, forming three plasmid groups according to sequence analysis. Homology to known plasmids present in typical strains of *A. salmonicida* was observed within the groups. The sequence data also indicated in five a*As* isolates exhibiting reduced antibiotic susceptibility, the presence of multi-drug resistance elements on plasmids capable of conjugal transfer. Together these findings help identify the prevalent strains of a*As* isolates causing disease in farmed wrasse in Scotland limiting cleaner fish deployment, and inform fish health management and future control measures.

Conference Session Designation: (Cleaner Fish Session)
Presentation Format: (Poster)
Student Presentation: (Yes)

Pathobiology of an *Exophiala* Sp. Disease Outbreak from Aquaculture Reared Lumpfish (*Cyclopterus lumpus*) in Newfoundland & Labrador, Canada

Nicole O'Brien [1*], David Groman[2], Matthew E. Saab[2], Jan Giles[2], David Overy[3], Ashley L. Powell[2], Danny Boyce[4] and Daryl Whelan[1]

[1] Department of Fisheries and Land Resources, Aquatic Animal Health Division, 30 Strawberry Marsh Road, St. John's, NL, A1B 4J6 Canada nicoleobrien@gov.nl.ca
[2] Aquatic Diagnostic Services, Atlantic Veterinary College, University of Prince Edward Island, Charlottetown, Prince Edward Island, Canada
[3] Ottawa Research and Development Centre, Agriculture and Agri-Food Canada, Ottawa, Ontario, Canada
[4] Dr. Joe Brown Aquatic Research Building (JBARB). Department of Ocean Sciences, Memorial University of Newfoundland, NL Canada.

Cleaner fish are used worldwide as a tool in an Integrated Pest Management Plan to mitigate sea lice in salmonid aquaculture. Lumpfish (*Cyclopterus lumpus*) and Cunners (*Tautogolabrus adspersus*) are used in salmonid aquaculture as cleaner fish. Lumpfish are favoured in the Newfoundland & Labrador aquaculture industry due to the short growing season. Lumpfish grown in an aquaculture setting reach sexual maturation in about 2 years and the males have a characteristic colouring allowing for easy identification. The females are stripped, eggs collected and the milt harvested post-mortem. The eggs are placed in a single layer on a specialized grid surface to enable the egg mass to be formed. By ~300°C degree days the eggs will hatch. Currently cleaner fish in NL are being vaccinated with a dip vaccine containing the antigens *Vibrio anguillarum* and *Vibrio ordalli*.

The current case study describes a population of 2015 year class Lumpfish that were hatched and raised at a land based sea water research facility in Newfoundland and Labrador. In January 2018, the population of lumpfish presented with clinical signs described as darkened skin lesions. The clinical signs consisted of systemic disease resulting in darkened and necrotic gills as well as dark internal organs, such as the heart. This population of fish were diagnosed with a fungal infection, identified as *Exophiala* spp. This mycotic class of organisms are ubiquitous in the soil and water and can affect finfish by causing local invasion or systemic disease. Diagnosis was initially determined by routine histopathology, with the aid of PAS staining to assist in identifying septate fungal hyphae in the affected organs and tissues. Subsequent confirmation was completed by culturing the infective isolate and submitting samples to a Canadian reference lab for morphologic and genomic identification. This talk will outline the epidemiology and pathology of the mycotic infection.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: **(** Oral)

Experimental Investigations Into Ranavirus (*Iridoviridae*) Infections in Lumpfish (*Cyclopterus lumpus*)

Felix L Scholz[1, 2*], Niccoló Vendramin[4], Hamish D Rodger[1], Neil M Ruane[3], David Swords[3], Cathy Hickey[3], Ian O'Connor[2], Luca Mirimin[2], Niels Jørgen Olesen[4] and Eugene MacCarthy[2]

[1] FishVet Group Ireland, Oranmore, Co. Galway, Ireland felix.scholz@fishvetgroup.com, hamish.rodger@fishvetgroup.com
[2] Marine and Freshwater Research Center, Galway-Mayo Institute of Technology, Galway, Ireland Ian.OConnor@gmit.ie, luca.mirimin@gmit.ie, eugene.mccarthy@gmit.ie
[3] Fish Health Unit, Marine Institute, Oranmore, Co. Galway, Ireland neil.ruane@marine.ie, david.swords@marine.ie, cathy.hickey@marine.ie
[4] DTU Aqua, National Institute of Aquatic Resources, Copenhagen, Denmark niven@vet.dtu.dk, njol@vet.dtu.dk

A ranavirus (*Iridoviridae*), closely related to the notifiable epizootic haematopoietic necrosis virus (EHNV), has been repeatedly isolated from lumpfish (*Cyclopterus lumpus*). Isolates from Scotland, Iceland and the Faroe Islands were not associated with clinical disease. In Ireland the virus was isolated from lumpfish fry experiencing high mortality, but to date the virus has not been proven to be the aetiological agent of the disease. However, histopathology was indicative of viral aetiology and no other pathogens were identified using histology, bacteriology or parasitology. Several ranavirus species can cause severe systemic disease in fish and show a low host specificity, raising concerns about potential biosecurity risks posed to cohabited Atlantic salmon (*Salmo salar*). Challenge trials were conducted to evaluate the virulence of the virus to lumpfish and Atlantic salmon. Initially, sea transfer size lumpfish and lumpfish fry were challenged by immersion, Atlantic salmon smolts were challenged by immersion and intra peritoneal (IP) injection with the Irish isolate. Infection was demonstrated in fry but results were considered inconclusive and a second trial was set up using a cohabitation model. In this model, lumpfish fry were injected with Irish, Icelandic and Faroese strains of the virus and cohabited with naïve lumpfish. Atlantic salmon juveniles were IP injected with the Irish isolate without cohabitation of naïve fish. This challenge model demonstrated replication of the virus in the lumpfish, horizontal transmission of the virus and reduced survival in the IP injected lumpfish. A ranavirus qPCR assay was used to monitor the viral load in shedders and cohabitants at set time points and in mortalities. Results will be presented.

This research is funded by the Irish Research Council (Employment Based Postgraduate Program), FishVet Group Ireland, in part by Bord Iascaigh Mhara and through Aquaexcel 2020.

Conference Session Designation: (Cleaner Fish Diseases)
Presentation Format: (Oral)
Student Presentation: (Yes)